β-Carbonic Anhydrase from *Pisum sativum*: Crystallization and Preliminary X-Ray Analysis

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Introduction: Carbonic anhydrases (CA) are enzymes that catalyze the reversible hydrolysation of CO_2 to bicarbonate. There are three different proteins with independent evolutionary origins, designated α, β- and γ-CA. β-CA in plants plays a role as facilitator of high turnover in the photosynthetic dark reaction.

Methods and Materials: β-CA from garden peas was cloned and over expressed in *Escherichia coli* cells. Purified protein was crystallized by vapour phase equilibration against 16% PEG4000, 0.4 M ammonium acetate, 50 mM DTT and 100 mM sodium citrate pH 5.0. Macroseeding drastically improved the quality of the crystals. From a single flash-frozen crystal, a native data set complete to 2.8 Å resolution was collected at the NSLS, beamline X8C using radiation of 0.9500 Å wavelength and a MAR345 area detector. All data were reduced using the HKL package.

Results: Crystals of β-CA from garden peas adopted space group C222, unit cell parameters a = 136.3 Å, b = 142.5 Å, c= 201.4 Å. They grew to 0.2 x 0.2 x 0.08 mm3 in size. Unit cell lengths, however, could vary up to 2% for different crystals. The crystals were also quite anisotropic in their scattering with diffraction limits varying between 2 Å and 4 Å. Self rotation analysis of the Patterson function revealed the presence of several weak two-fold rotation axes but no four-fold axis was found.

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